SOME CHEMICAL AND MORPHOLOGICAL PHENOMENA ATTENDING INFECTION OF THE WHEAT PLANT BY OPHIOBOLUS GRAMINIS [1]

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INTRODUCTION

Take-all of wheat, caused by *Ophiobolus graminis* Sacc., was first discovered in the United States in Virginia in 1919. Since that time it has been found in most of the winter-wheat areas of this country. During the last few years some work has been, done on the disease and the causal fungus by Davis (*2*),[2] Kirby (*3*), McKinney and Davis (*4*), Webb and Fellows (*11*), and others in the United States. In Australia considerable attention has been given to take-all, and in France some work has been done on what is thought to be the same disease. However, Davis (*2*) is the only worker who has studied the microscopical and macroscopical phases of infection. The nature of Davis's work is briefly described in his summary as follows:

> Histological studies were made from plants exhibiting various stages of infection and these showed that the parasite entered the unbroken epidermis of the underground portions of the leaf sheaths, culms, and roots. The parasite first destroys the cortex of the roots and later enters the central cylinder. It also destroys the leaf-sheath and culm tissues and later enters the vessels, but it does not appear to make much progress after it enters the vessels.

The work on infection described in the present paper has been of two kinds, (1) morphological and histological and (2) microchemical. The purpose of the histological study was to learn what tissues were invaded and what morphological changes occurred in these tissues. The composition of the cell walls before and after infection was determined by microchemical methods. The roots, subcoronal internode, and crown were used in the histological study, but only the roots were employed in the microchemical studies. The results of the work described in this paper already have been a valuable aid in interpreting the behavior of wheat plants affected with take-all.

MATERIALS AND METHODS

Most of the histological studies were made with material embedded in paraffin. A combination of safranin and light green was found to be the best stain for differentiating the host and the parasite. This method of staining was especially satisfactory in lignified tissues where the host cell walls stained red and the fungus green.

Phloroglucin, used according to the method given below, was the best reagent for the detection of the fungus in the cell wall itself. Thin sections of fresh tissue were first treated 10 minutes in hot

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[2] Reference is made by number (italic) to "Literature cited," p. 661.

hydrogen peroxide with frequent changes. The sections were then transferred to a 1 per cent solution of alcoholic phloroglucin on a glass slide and allowed to remain until the solution had mostly evaporated. One or two drops of concentrated hydrochloric acid then were applied, the cover slip placed in position, and the sections observed. Lignified cell walls showed red after this treatment, whereas the fungus hyphae passing through the walls showed as dark lines.

Kanred wheat was used in all of the studies described in this paper.

Where constant temperatures were maintained, soil-temperature control tanks, of the type developed in Wisconsin, were used.

Microchemical tests were made according to mimeographed directions received from Sophia H. Eckerson of the Boyce Thompson Institute for Plant Research (Inc.), Yonkers, N. Y. Other supplementary references were used (*1*, *9*). Briefly, some of the principal microchemical tests employed were as follows:

CELLULOSE: (1) Stain.—Iodine-potassium iodide+75 per cent sulphuric acid. (2) Solubility.—Copper oxide ammonia, 50 per cent chromic acid, zinc chloride and hydrochloric acid about 1:2.

HEMICELLULOSE: (1) Stain.—Furfural reaction with 1 per cent phloroglucin in alcohol and concentrated hydrochloric acid+heat; 4 per cent orcin and concentrated hydrochloric acid+heat. (2) Solubility.—Hot 3 per cent sulphuric acid.

CALLOSE: (1) Stain.—Resorcin blue (1:1,000), aniline blue dilute solution. (2) Solubility.—Cold 1 per cent sodium hydroxide or potassium hydroxide.

PECTIC SUBSTANCES: (1) Stain.—Ruthenium red (1:10,000 in water); methylene blue (1:1,000 in water). (2) Solubility.—Two per cent hydrochloric acid changes pectose to pectin or pectic acid; 2 per cent ammonia dissolves pectic acid.

LIGNIN: (1) Stain.—One per cent phloroglucin in alcohol+25 per cent hydrochloric acid; phenol-potassium chlorate mixture followed by concentrated hydrochloric acid. (2) Solubility.—Fifty per cent chromic acid. (3) Oxidation.—Hydrogen peroxide, potassium chlorate.

FURFURAL: (1) Stain.—Furfural reaction. (2) Removal.—Hydrocyanic acid.

SUBERIN: (1) Stain.—Sudan III. (2) Solubility.—Three per cent alcoholic potassium hydroxide. Insoluble in 50 per cent chromic acid.

MORPHOLOGICAL AND HISTOLOGICAL STUDIES

PRIMARY ROOTS

DESCRIPTION

The primary roots originate from the hypocotyl of the embryo and are the roots upon which the young plant depends for the intake of water and mineral nutrients before the crown and secondary or permanent roots arising from it are formed. The primary roots do not necessarily end their usefulness to the plant when the permanent roots are formed. Their duration as useful organs after this time varies according to conditions. Sometimes the primary roots may still be functioning when the plant is mature. On the other hand, many of the primary roots may die and disintegrate soon after the secondary roots are formed.

A cross section of a young primary root shows it to be composed generally of three parts—epidermis, cortex, and stele. (Fig. 1.) The epidermis consists of a single layer of elongated thin-walled cells. It is from these epidermal cells that the root hairs are developed and it is through the epidermal cells that infection occurs. The cortex lies just beneath the epidermis and is composed of four or five layers of large, thin-walled cells. The endodermis, the innermost layer of

the cortex, consists of a single, continuous layer of closely fitting cells. The outer tangential walls of the endodermal cells are thin, while the radial and inner tangential walls are thickened.

The pericycle, the outermost layer of the stele, consists of a single layer of radially elongated cells. The walls of the pericycle cells are slightly thickened. All the cells are nearly equal in size, except those opposite the xylem, which are smaller.

The xylem strands are seven or eight in number and alternate with the phloem. The protoxylem vessels are strengthened with spiral thickenings, but those formed later toward the center are pitted. The phloem bundles consist of series of three thin-walled cells. The conjunctive tissue of the stele consists of thin-walled irregular parenchyma cells. In the center of the stele is found a large central vessel with a heavy wall.

Infection

It is well to mention here that the hyphae of *Ophiobolus graminis* are of two kinds, distinguished by color and size. The macrohyphae are large in diameter, thick walled, and dark in color. The microhyphae are more slender, thinner walled, and colorless. The macrohyphae are ordinarily, though not always, found on the outer surface of tissues, and the microhyphae are generally found within the cells.

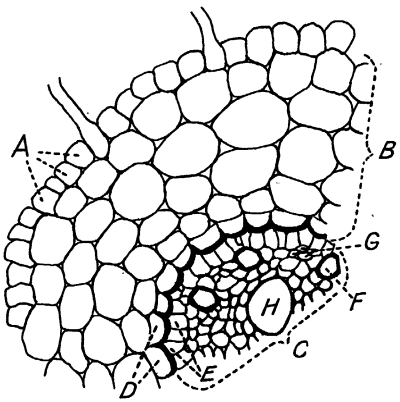


FIG. 1.—Sector of a cross section of a healthy primary root of a wheat plant. A, Epidermis; B, cortex; C, stele; D, endodermis; E, pericycle; F, xylem; G, phloem consisting of groups of three cells; H, central cavity. × 231

The macrohyphae, as they are ordinarily found on the roots, grow in contact with the epidermal cells. Where the macrohyphae are thus in contact with the epidermal cells, microhyphae may branch off at any point and penetrate the epidermal cells of the roots. The original penetration from the outside may or may not be attended with constrictions of the hyphae at the point of entrance. Once inside the root, the hyphae seldom undergo a change in size when passing through a cell wall except in instances where the cell wall undergoes a change in the immediate presence of such hyphae. These changes will be described later in this paper.

After entering the cortex the hyphae grow intracellularly from cell to cell in a radial or obliquely radial direction. In the cells of the cortex they have never been observed to parallel the long axis of the root. While the endodermis offers some resistance to the radial growth of the fungus, various segments of it differ in this respect. Certain portions may offer no resistance and others much. Finally the hyphae enter the stele and may penetrate any of its cells. A small lesion on one side of a root is sufficient to permit all of the vital conducting regions of the stele to become infected. The fungus that has entered one side of a root and has penetrated into the stele has never

been observed to pass out of the stele into the cortex on the side opposite the primary infection.

After entry into the stele the hyphae show a tendency to grow lengthwise of the root. This occurs especially in the xylem tubes or in the spaces created by the disintegration of the parenchyma and phloem of the stele.

It is well to mention here that a short time after complete or incomplete occupancy of the root the hyphae die and disintegrate completely. Therefore, the only indications of the earlier presence of the fungus are the malformations on the remaining cell walls or the destruction of cell contents and cell walls. The stele may or may not have been entered before this disintegration occurred.

The foregoing description may give the impression that the advance of the fungus through the invaded tissue is always fairly regular, but such is not invariably the case. In some cells the hyphae become aggregated much more than in others. The wall of a cell thus filled with mycelium becomes greatly thickened, making it difficult for the hyphae to pass from it to the next cell. In other cases the progress of a single hypha is in nearly a straight line so that it may be traced through four to five cells in succession. These variations in the behavior of the fungus may be due either to differences in food supply in the different cells or to mechanical or other differences in the nature of the cell walls themselves. In the former case the hyphae may remain and branch abundantly in the cells where the food is abundant, while in the latter case the hyphae may find it difficult to escape from some cells and thus be forced to remain. Aggregations of hyphae occur not only in single cells but also in groups of adjacent cells. Judging from the variations in behavior of the fungus in different cells, it seems that cells of the same tissue differ individually.

SECONDARY ROOTS

Description

The secondary roots, which form the bulk of the root system, are formed at or near the crown of the plant. Their number is not limited to a few (usually three), as is true of the primary roots. The crown is located at or somewhat below the surface of the ground and is the region in which several nodes are closely crowded together and from which tillers and leaves as well as secondary roots are formed. Secondary roots are formed not only in the original culm of the plant, but also grow from basal nodes of the tiller culms. New secondary roots may be forming for a considerable time during the early growth of the wheat plant.

The anatomy of the secondary roots does not differ greatly from that of the primary, except that their diameters are greater because of the greater quantity of parenchymatous tissue in the cortex and more abundant conjunctive tissue in the stele. As a secondary root becomes older, the walls of the outermost two or three layers of cortical cells become thickened. This thickening occurs first at the base of the root, that is, in the older portion, and gradually progresses toward the tip.

Infection

Infection and progressive penetration of the secondary roots by the fungus do not differ materially from those of the primary roots, with one exception. Within the thick-walled cortical cells, mentioned above, and in some of the cortical cells immediately outside of these, the hyphae become aggregated in large numbers, assume the form of macrohyphae, and grow lengthwise in the cells. These macrohyphae may become so numerous in a cell as to cause it to bulge and break. Furthermore, by means of microhyphae, the macrohyphae may penetrate outward through the cell wall, in which they are inclosed, in any direction rather than only toward the center of the root.

COLEOPTILE

Description

The coleoptile forms a complete sheath for the young growing plumule, its only opening being a slit near the apex. It is in reality a leaf folded longitudinally on itself with the two margins united. Within the cavity of the coleoptile is located the plumule.

In cross section the coleoptile is oval. At each extremity in the oval is a vascular bundle. The outer epidermis is composed of small cells more or less radially elongated and with their outer tangential walls slightly thickened. The cells of the inner epidermis, which are in more or less direct contact with the plumule, are similar to the cells of the outer epidermis except that they are elongated tangentially and the walls are not thickened. The tissue between the outer and inner epidermis is composed of large parenchyma cells with the exception of the two vascular bundles mentioned above. These parenchyma cells will be called in the present paper mesophyll. (Pl. 1, B.)

Infection

As the coleoptile becomes infected, macrohyphae are found closely appressed to the outer epidermis with their long axes parallel to the long axis of the coleoptile. These hyphae may be piled on one another many layers deep. Now and then a macrohypha ends off a side branch toward the epidermis. At the junction of the attacking hyphae and the attacked cell the hyphae may still be macrohyphae. As penetration of the host cell wall is effected a marked constriction occurs in the penetrating hypha. After emergence on the inner side of the wall the hypha may or may not enlarge somewhat.

Penetration continues through the mesophyll to the inner epidermis. When the inner epidermis of the coleoptile is reached by the hyphae they accumulate in great abundance in that vicinity and apparently are unable to proceed farther. Sometimes the inner epidermis may be infected, but more often it is not. The writer has never seen a case where the hyphae have penetrated the young leaves from the coleoptile, nor observed any lesions on the leaves as they emerge from the coleoptile. Seemingly the coleoptile acts as a protective organ for the inclosed young plumule during its early stages. A similar kind of protection has been noted by Reed and

Melchers (*7*) with respect to the attack of milo and feterita seedlings by *Sphacelotheca sorghi*.

It is true that the subcoronal internode, which often is infected, is surrounded by the coleoptile. However, the coleoptile has become ruptured by growth processes and often is mostly disintegrated and therefore can offer no further protection.

SUBCORONAL INTERNODE

DESCRIPTION

The subcoronal internode is the underground part of the stem of the wheat plant, below the crown. It is called a rhizome by Percival (*5*). On its upper end is the crown, and at its lower extremity are the primary roots and the remains of the seed.

A view in cross section shows the subcoronal internode to consist of essentially four parts, (1) epidermis, (2) cortex, (3) a cylinder of vascular bundles, and (4) the pith in the center. (Fig. 2.) The epidermal cells are closely appressed to one another and are consequently more or less square in cross section or elongated radially. The outer and radial walls are heavily lignified. The cortex has a thickness of six or seven layers of cells, the outer cells small, but gradually increasing in size toward the center. These cells are not especially thick walled. Occasionally there are groups of sclerenchyma cells in the cortex.

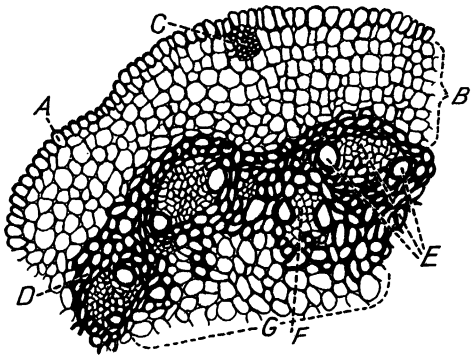


FIG. 2.—Portion of a cross section of the subcoronal internode of a healthy wheat plant. A, Epidermis; B, cortex; C, group of sclerenchyma cells; D, groups of thick-walled cells connecting with one another and making a complete cylinder surrounding the bundles and pith; E, large bundle; F, small bundle; G, pith. × 96

The bundles are arranged in a circle of seven large ones alternating somewhat irregularly with seven smaller ones. Each bundle is surrounded by thick-walled lignified cells, which are joined from bundle to bundle, thus forming a continuous irregular circle inclosing all the bundles and the pith. The pith is composed of large, thin-walled parenchyma cells loosely joined together.

INFECTION

Infection of the subcoronal internode is similar to that of the roots; that is, infection occurs through the epidermis, at any point. The hyphae proceed though the cortex and bundle sheaths into the pith. Any cell may become infected. The hyphae, after entering the xylem tubes and thick-walled cells of the bundle sheaths, assume the form of macrohyphae and grow lengthwise in these cells. This lengthwise growth of a single hypha has been traced as far as 1 cm. and doubtless may extend farther.

CROWN

The primary purpose of the present study of the crown was to learn the behavior of the crown and of the fungus in the crown of plants that were able to recover from the take-all disease. The crown is of particular interest from this standpoint because in such plants it has been only partially invaded. In a recovered plant the primary roots, the subcoronal internode, the first, second, and third leaves, and some of the secondary roots, expecially the lower ones, all may be destroyed, while the upper part of the crown and the higher secondary roots may be free from the disease. Such recovered plants are able to grow to maturity and produce a normal head. As pointed out by Davis (*2*), such upper secondary roots no doubt may escape infection on account of their later formation. The crowns of recovered plants are infected only in part. As soon as infection becomes general in the crown the entire plant dies and no recovery is possible.

DESCRIPTION

The crown is the region in which the tillers, leaves, and secondary roots are formed. (Fig. 3.) The elongation of the culm, however, carries some of the leaves up away from the crown. The crown is surrounded by the sheath of the first leaf and partially also by the sheaths of the other lower leaves.

The lower true leaves and secondary roots, which latter usually come in pairs, alternate with each other vertically on the stem. The first true leaf comes at the junction of the subcoronal internode and the crown. The first two secondary roots come just above the attachment of the first leaf, one on each side at points about 90° from the middle of the base of the leaf. In growing outward each of these two secondary roots penetrates the base of the first leaf and thus ruptures it at two points. The second and other successive lower leaves alternate with the second and other pairs of secondary roots in a similar manner. Thus the leaves are two ranked on the stem, and the second and other successive pairs of secondary roots come approximately at right angles to each preceding pair.

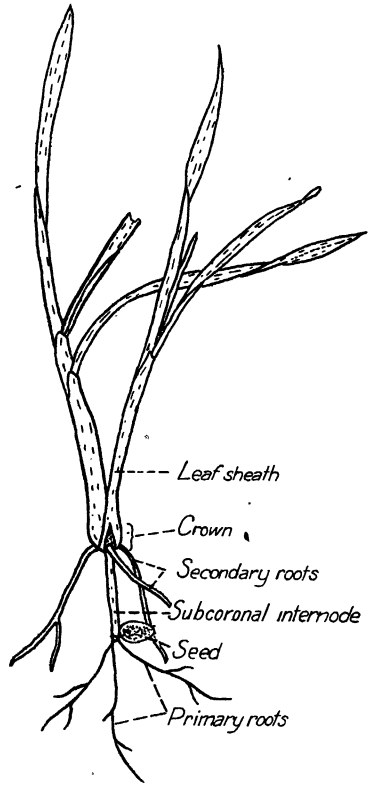


FIG. 3.—Diagram of a young wheat plant illustrating the location of the various parts. Subcoronal internodes may vary greatly in length

The first, second, and third leaves receive their vascular traces directly from the bundles found in the subcoronal internode. The

trace of the fourth leaf, however, does not connect directly with the vascular strands found in the subcoronal internode, but with branches of these. These branches come nearly at a right angle and meet the traces of the fourth leaf near the center of the crown. The fourth leaf, however, does not depend entirely upon the branches of the bundles from the internode for its supply of water and nutrients, for it is also connected with the vascular systems of the second pair of secondary roots. Subsequent leaves are connected with the vascular systems of the second and third pairs of secondary roots by means of branches of bundles sent from these roots to near the center of the crown where they connect with the leaf traces.

As pointed out above, the first two secondary roots come from the crown just above the attachment of the first true leaf. Their vascular systems connect directly with the established system coming from the subcoronal internode. The vascular systems of the second pair of secondary roots also connect directly with the bundles from the subcoronal internode and also establish an independent vascular system extending up the culm. This independent system branches toward the center of the crown, connecting with the fourth and fifth leaf traces and extending into the apical bud.

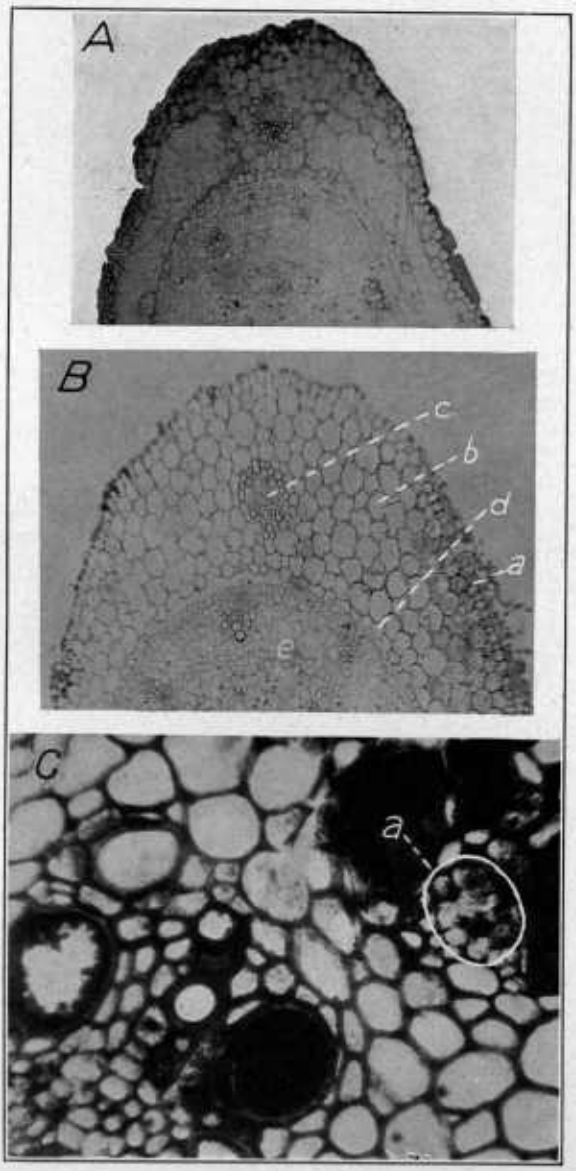
The vascular systems of the third pair of secondary roots have no connection with the bundles from the subcoronal internode but are independent and branch toward the center of the crown, connecting with leaf traces and extending to the apical region.

Infection

As noted above in discussing the infection of the subcoronal internode, the parenchyma cells of the cortex may become infected. There is an extension of these parenchyma cells into the crown for a short distance, but for some reason there is a sharp line of demarcation between the infected cells in the subcoronal internode and the uninfected cells in the crown. The parenchyma cells of the pith also extend into the crown. These cease in the region where the vessels from the subcoronal internode branch toward the center to connect with the traces of the fourth leaf. The latter parenchyma cells are infected as far as they extend into the crown but no farther.

If one cuts a longitudinal section in a median plane of the crown of a recovered plant, this pith region with its darkened infected cells may be seen to extend into the crown in a cone-shaped area. The apex of the cone is just below the branches of the bundles from the subcoronal internode to the traces of the fourth leaf.

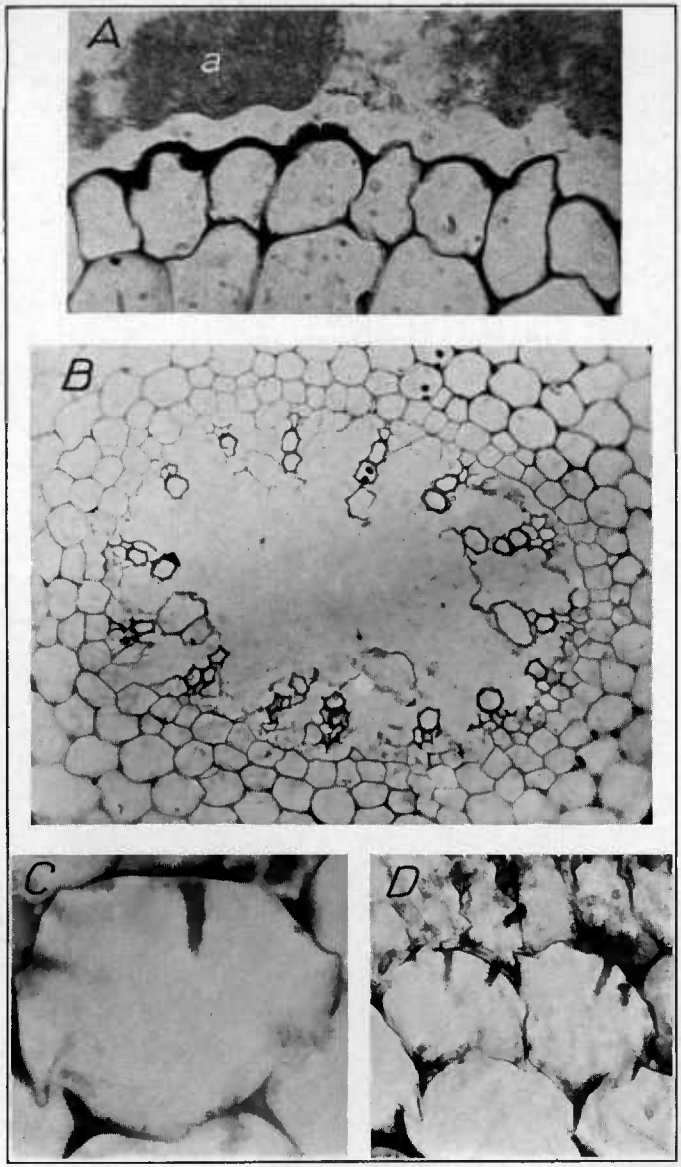
The hyphae in the cortical cells of the subcoronal internode extend out into the parenchyma cells of the first leaf. The hyphae growing lengthwise in the xylem cells of the subcoronal internode grow up into the xylem of the first leaf and to some extent may pass into the vascular strands of the first pair of secondary roots. It is noticeable that the hyphae become fewer and fewer in the xylem strands as the top of the subcoronal internode is approached. This diminution is more marked as they enter the crown. The same is true of the hyphae that enter the crown through the secondary roots. The reason for this will be discussed under a separate heading.



A.—Portion of a cross section of an infected coleoptile in which the mesophyll is disintegrated. The outer epidermis and most of the first layer of cells beneath it, the inner epidermis, the bundle, and the cells in its vicinity are not disintegrated. × 57

B.—Portion of a cross section of an infected coleoptile in which the cells of the mesophyll are not disintegrated: *a*, Outer epidermis; *b*, mesophyll; *c*, one of the two fibrovascular bundles; *d*, inner epidermis; *e*, leaves of the plumule. × 67

C.—Portion of infected vascular region of the subcoronal internode. The xylem tubes have become partly or completely clogged by products from their own walls; *a*, disintegrated phloem. × 500



A.—Portion of outer epidermis of the coleoptile showing thickening of outer tangential walls of epiderma cells caused by the incrusting layer of hyphae; the epidermal cells are not penetrated. The layer of hyphae *a* had been somewhat pulled away from the epidermis in the preparation of the section. × 330

B.—Stele of a primary root largely disintegrated by *Ophiobolus graminis*, with the xylem tubes and some of the parenchyma and pericycle cells in their immediate vicinity still intact; the walls of the endodermis have become thin. × 400

C and D.—Lignitubers in the cortical cells of roots of young wheat plants. × 1,000

MORPHOLOGICAL CHANGES IN CELL WALLS IN BASAL PORTIONS OF INFECTED PLANTS

Changes in the Roots

The morphological changes in the cell walls of the primary and secondary roots are very similar and will be discussed together. Changes that occur in the wall of one cell may be very different from those that occur in another, although the two cells may be comparatively close to each other, of a similar type, and have equal quantities of hyphae in them. Accordingly, the description of a change in a certain cell wall can not apply to the walls of all infected cells of that particular type.

Shortly after or even before the entry of mycelium into a cell its wall may become very much thickened. This thickening may or may not be uniform on all surfaces of the wall. It is especially noticeable at the corners. The thickening is not necessarily formed on the side of the wall toward the lumen, for it may form between the cells, or the intercellular spaces may be filled with it.

Somewhat similar cell-wall thickenings in diseased plant tissues have been observed by other investigators. Ravn (*6, p. 113*) described and figured cell-wall thickenings in oats attacked by *Helminthosporium avenae* Eidam. The composition of these thickenings was not definitely determined, although it was stated that they stained intensely with thionine and other basic stains.

Tisdale (*10*), working with flax wilt, pointed out that certain cell walls of the flax plant may become thickened in advance of the hyphae of *Fusarium lini* Bolley, and he attributed the thickening to the formation of suberin.

When a wheat root becomes infected with *Ophiobolus graminis* very noticeable changes occur in the walls of the infected cells in some cases. When an infecting hypha starts to penetrate a cell wall a slight protuberance is formed on the cell wall at a point just opposite. The protuberance elongates at right angles to the wall, becoming longer and longer in front of the hypha as the latter advances. (Pl. 2, C and D.) As the hypha progresses it becomes more and more attenuated. Finally either the hypha is able to outgrow the protuberance and enter the lumen of the cell or the protuberance is able to prevent its doing so. If the hypha passes through the protuberance, the hypha immediately increases in diameter to normal size again.

Protuberances in wheat cells caused by other organisms but similar to those described above have been observed by others, and the names callosities and calluses have been applied to them (*8, 12*). Either of these names would be a misnomer if applied to the abnormalities produced in wheat by *Ophiobolus graminis*, as microchemical tests have shown that the protuberances here described contain no trace of callose but rather are composed chiefly of lignin. Therefore the writer suggests the name "lignitubers" for the protuberances caused by *O. graminis*, the name alluding to their composition and form. This designation will be used in the present paper.

In form the large lignitubers have the general shape of a finger. (Fig. 4.) The surfaces are generally smooth and slightly undulating. The bases flare out and are joined to a similar substance on the inner surface of the cell walls. The same substance may occur also between the walls of the adjoining cells where the hypha penetrates. When the

lignituber is viewed sideways a lighter staining median line may be seen extending lengthwise through or almost through it. This line is the hypha contained within. When viewed in cross section a lignituber resembles a doughnut, the central hypha corresponding to the hole in the doughnut. In size these lignitubers may vary greatly. Some are very minute; others are sufficiently long to cross completely the cell lumen.

Lignitubers do not invariably accompany penetrating hyphae. They are usually found most abundantly on walls of cells that thicken their walls otherwise in the presence of the attacking organism, and they may occur in cells of all the different tissues of the root.

In part of the cortex of the proximal portion of the secondary roots, about 0.5 cm. from the crown, it was found that the hyphae after entering certain cells often grew lengthwise rather than always penetrating the cells crosswise—that is, in the radial direction—as was the usual procedure in portions of the roots farther from the crown. In seeking an explanation for this behavior of the hyphae in those particular cortical cells close to the crown the corresponding tissues of a healthy root were examined. It was found that there occurred in the cortex of the root, near the crown, at a depth of one or two layers of cells below the epidermis, a ring of cortical tissue with rather thick cell walls. This ring was three or four cells in thickness. It seemed that the ability of these cortical cells to thicken their walls might account for the rather peculiar behavior of the hyphae in this tissue. This might occur in two ways. Hyphae that were in these cells when the thickening was taking place found difficulty in escaping radially and as a result grew lengthwise in the cells; or these cells, because of their inherent ability to thicken, were able to produce thick walls quickly, due to the stimulation of the fungi within, and thus forced the hyphae to grow lengthwise in the cells.

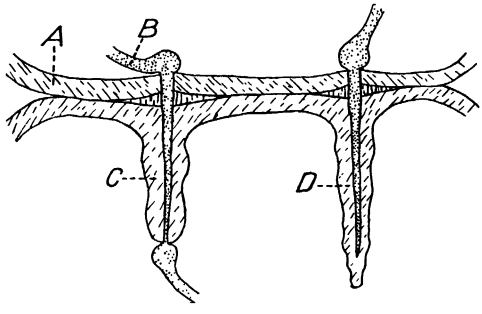


FIG. 4.—Diagram showing penetration of cell walls by infecting hyphae and lignitubers formed about them. A, Cell wall; B, hypha; C, lignituber through which the hypha has passed; D, lignituber through which the hypha has not passed. (See pl. 2, C and D.) × 3000

After the stele of either the primary or the secondary roots becomes invaded a disintegration of certain of the cells within may occur. When this happens the phloem and most of the parenchyma of the stele disintegrate, the phloem usually more rapidly than any of the other tissues. The walls of the xylem vessels, as well as the walls of the parenchyma and pericycle cells relatively close to the xylem, remain intact. (Pl. 2, B.) Such disintegration does not always occur, but it usually does. In cases of such disintegration the distal portion of the root beyond the lesion, of course, functions no longer.

Another notable change occurring in the bases of such infected secondary roots is the clogging of the xylem vessels and sometimes of the surrounding cells. The walls of the xylem vessels may become thickened to such an extent that the lumen often is entirely filled.

Changes in the Coleoptile

In a preceding paragraph it was mentioned that the macrohyphae accumulate in large numbers on the outer epidermis of the coleoptile. The presence of these hyphae, before they penetrate the cell walls of the epidermis, causes these walls to become greatly thickened with lignified material. (Pl. 2, A.) This thickening is most pronounced on the outer, tangential walls, but it also extends down the radial walls. When penetration of the epidermal wall occurs the hyphae apparently are always accompanied by lignitubers, many of which are larger than those seen in any other cells. The row of cortical cells immediately below the epidermis reacts similarly but not to such a marked degree. The walls of the other cells of the coleoptile mesophyll neither become thickened nor form lignitubers in the presence of the hyphae. The cell walls of the inner epidermis become slightly thickened but apparently do not form lignitubers.

Comparatively soon after infection the mesophyll cells of the coleoptile disintegrate completely with the exception of those near the two bundles. After this disintegration the two empty spaces thus formed appear as two crescents with their concave sides toward each other. The two bundles with the immediately surrounding mesophyll separate the two ends of these crescents. (Pl. 1, A.)

Changes in the Subcoronal Internode

As stated previously, in a healthy plant the outer tangential walls of the epidermal cells are thickened. Apparently no additional thickening occurs after penetration by the fungus. On the other hand, the cell walls of any or all of the cells of the cortex may become thickened after penetration. This thickening is especially noticeable in the intercellular spaces. These become completely filled with a substance staining strongly with safranin.

The greatest changes that were noticed after infection were in the xylem tubes and some of the thick-walled cells surrounding the bundles. It was found that the walls of these cells became thickened to such an extent that the lumen became entirely filled. Most of the xylem tubes became completely plugged. This plugging occurred in advance of the hyphae or when they were present. (Pl. 1, C, and fig. 5.) In the latter case it was noticeable that the hyphae became disintegrated. Undoubtedly such plugging hinders considerably the upward progress of the hyphae into the crown. In this way the plant may be helped to recover from the disease, provided secondary roots are formed in large enough numbers to provide for the needs of the young plant after the primary roots have been cut off by the plugging of the vessels leading through the subcoronal internode.

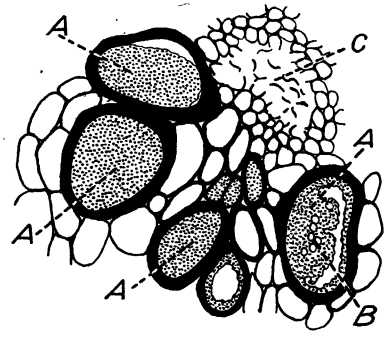


FIG. 5.—A diseased vascular bundle of the subcoronal internode. A, Xylem tubes that have become clogged or partly clogged with an outgrowth of their own walls, some of the smaller cells in the vicinity having also become clogged or partly clogged; B, a group of hyphae that are surrounded by the clogging material within a xylem tube; C, disintegrated cells of the phloem region. (See pl. 1, C.) × 400

As was the case in the roots, the phloem of the bundles in the subcoronal internode, as well as the pith, often disintegrates in the presence of the parasite.

Changes in the Crown

As previously noted, studies of the crown have been made only on plants that have recovered from the disease. In such plants the hyphae have entered the crown through the xylem tubes of the subcoronal internode and lower secondary roots or through the pith of the subcoronal internode. The progress of the hyphae entering through the xylem tubes is so much hindered by the great thickening of the walls and subsequent plugging of these vessels that further progress in some tubes may be stopped. The hyphae entering through the pith disintegrate this tissue in the crown to a point just below where the vessels from the subcoronal internode branch off to the fourth leaf. The further progress of these hyphae is probably hindered by the rather thick, relatively resistant cell walls in the region of the branching.

MICROCHEMICAL STUDIES OF HEALTHY AND DISEASED ROOTS

As previously noted, infection of the basal portions of the wheat plant by *Ophiobolus graminis* is accompanied by certain morphological changes. Certain cell walls become thickened, and peculiar outgrowths called lignitubers are formed. It was the object of the microchemical studies, the results of which are here summarized, to determine what chemical changes, if any, occurred in the walls of the infected cells, (1) where morphological changes were evident and (2) where no morphological changes were noticeable.

The plants used in these studies were, on an average, 9 days old. They were grown in steamed soil subsequently infested with a pure culture of *Ophiobolus graminis* isolated from wheat in Kansas. The infested soil was placed in large test tubes, and disinfected seed of Kanred wheat was sown in it. These tubes were then kept at a temperature of about 20° C., and the moisture of the soil was kept at about 55 per cent of its moisture-holding capacity. The results are given in Table 1.

TABLE 1.—*Comparison of cell-wall constituents of healthy and diseased primary and secondary roots of young Kanred wheat plants grown in large test tubes at about 20° C.*

Cell-wall constituents in healthy roots	Cell-wall constituents in diseased roots
CELLULOSE	
All of the cell walls, including those of the root hairs, had their foundations of cellulose.	In general some cellulose was present in all cell walls, but it varied more in diseased than in healthy roots. Many cell walls of the cortex had lost all or part of their cellulose. The endodermis and parenchyma of the diseased stele had less cellulose than the healthy material. The lignitubers had no cellulose so far as tests showed. The pericycle of the diseased plants had little cellulose in comparison to that of healthy plants.
HEMICELLULOSE	
The only form of hemicellulose present was pentosans, found in the xylem.	No change was produced by disease.
CALLOSE	
The test for callose was negative.	None was found. Evidently the disease did not cause its production.
PECTIC SUBSTANCES	
Pectose was the only pectic substance present. It was located in the root hairs and epidermal cell walls.	No change was produced by the infecting organism.
LIGNIN	
Lignification occurred only in the walls of the xylem tubes.	The walls of the cortical cells that were thickened in the presence of the organism showed large amounts of lignin and comparatively little cellulose. The lignitubers and the substance filling the intercellular spaces in the cortex were largely lignin; likewise some striations in the inner tangential walls of the endodermis showed lignin. The parenchyma of the pith and the pericycle near the xylem had become lignified.
SUBERIN	
Suberin was present in the xylem and radial walls of the endodermis. In the xylem it was located in the thickenings.	A slight amount of suberin had been produced in the thickenings of the infected walls of the cortex. There was also a slight quantity in the lignitubers. Neither the cell wall thickenings nor the lignitubers were completely soluble in chromic acid, but they were mostly so.

SUMMARY

The studies here reported pertain to infection of the wheat plant by *Ophiobolus graminis* Sacc., the cause of the disease known as take-all. It has been shown which of the tissues studied are invaded and what are the morphological and chemical changes produced by such invasion. The microchemical studies here reported were made on the roots only.

The behavior of the primary and secondary roots in the presence of the parasite is very similar. The fungus penetrates the epidermis, passes through the cortex, and enters the stele. Its progress is hindered to some extent by the endodermis.

Many of the cell walls with which the parasite comes in contact become thickened, or the walls may produce elongated protuberances in front of and around the invading hyphae. These previously undescribed structures are given the name lignitubers.

All the cells and vessels of the stele, except the xylem tubes and some cells near it, may be distintegrated after penetration.

Microchemical studies made on diseased roots showed that the disease caused a nonuniform reduction of cellulose, which was replaced by lignin and a slight amount of suberin. This is especially true of the thickened cell walls and the lignitubers. Other substances are not changed by the disease.

The coleoptile is penetrated through the unbroken epidermis, which is greatly thickened, and many lignitubers are formed in its cells. During the progress of the hyphae inward the mesophyll is destroyed except that near the bundles. The inner epidermis stops the further progress of the fungus and thus the coleoptile protects the young seedling, at least to some extent.

The subcoronal internode is attacked through the epidermis and may become infected in all parts. The pith and the phloem may become disintegrated. The xylem tubes and cells in their vicinity respond to the presence of the organism by thickenings on their inner walls by which they may be filled completely.

Studies of the crown were made on plants that had recovered from the disease. In these plants the lower part of the crown and the lower secondary roots were found to be injured. The hyphae had entered the crown through the pith and xylem tubes of the subcoronal internode and the xylem of the lower secondary roots. In many cases the xylem tubes where they enter the crown were plugged by excessive thickenings on their inner walls.

The results here reported have been found useful in interpreting the behavior of wheat plants affected with take-all and may have a bearing on the explanation of resistance, if resistant plants are found.

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